IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant	:	Oda, et al.
Appl. No.	:	10/501,691
Filed	:	July 16, 2004
For	:	METHOD FOR IMMOBILIZING BIOMOLECULE ON CARRIER

DECLARATION UNDER 37 C.F.R. § 1.132

Commissioner for Patents
Alexandria, VA 22313-1450

Dear Sir:

I, Naoki KIMURA, a citizen of Japan, of c/o Nisshinbo Industries, Inc., R&D Center, 1-2-3, Onodai, Midori-ku, Chiba-shi, Chiba, hereby declare and state as follows.

1. I graduated from the Graduate School of Hokkaido University, and was granted a doctorate degree of Science.

2. I have been employed by Nisshinbo Industries, Inc. since June 1998, and received an appointment to the Nisshinbo Industries, Inc., R&D Center.

3. I am engaged in research in the fields of bioorganic synthetic chemistry and macromolecule chemistry.

4. I am one of the inventors of the present invention. I have read and understood the specification of the present application, and the Office Action dated June 15, 2006.

5. The following experiments were conducted by myself.

6. **Materials and Methods**

Synthesis of oligonucleotides

An oligonucleotide (5'-GATTCGTGGTATGTGGGTAAC-3') labeled with 5'-hexachlorofluorescein (HEX) was synthesized on an ABI3900 DNA synthesizer at a 0.2 μmol scale using the standard phosphoramidite method, and then purified by reverse-phase HPLC

using a standard procedure and dried *in vacuo*. A non-modified oligonucleotide (the same sequence as the labeled oligonucleotide) was also synthesized on an ABI3900 DNA synthesizer at a 0.2 μmol scale using standard procedures. The non-modified oligonucleotide was then purified on a reverse-phase cartridge following a standard procedure and dried *in vacuo*.

Fabrication of DNA microarrays

A Pixsys DNA microarray spotter (Cartesian technologies) with a SMP10B pin (TeleChem International Inc.) was used to print arrays on poly carbodiimide-coated glass slides (Nisshinbo Industries Inc.). Prior to printing, 5'-HEX-labeled oligonucleotide was mixed with the non-modified oligonucleotide (ratio of 1:100, mol/mol) to prevent optical quenching. The mixture of the non-modified oligonucleotide and the HEX-labeled oligonucletoide in 3 x SSC (20 pmol/μl, total volume 20 μl) was printed onto arrays, in diameter of ~250 μm and at distances of 600 μm center-to-center. The humidity was maintained around 70% during printing. After printing, the arrays were irradiated (total 0.6 J/cm^2) using a CRM-FA Spectro Irdadiator (JASCO, Tokyo, Japan) with central irradiation wavelengths of 227, 254, 280, 307 and 334 nm with a range of ±15 nm (i.e. 212 ~ 242 nm, 239 ~ 269 nm, 265 ~ 295 nm, 292 ~ 322 nm and 319 ~ 349 nm), respectively. The arrays were then treated with a blocking buffer (3% BSA, 0.2M NaCl, 0.1M Tris-HCl (pH 7.5) and 0.05% triton-X100), washed with TE buffer (pH 7.2) for 5 min, and dried for storage. All procedures were performed at room temperature.

Signal detection and data analysis

The arrays were imaged on a Scan Array 4000 unit (Packard Biochip) with 10 μm resolution. A Cy3 optical filter was used during imaging of the arrays. The laser power and photomultiplier tube voltage were set to 100% and 60% for the Cy3 channel. Analysis of the intensity of the original 16-bit tiff images from the Cy3 channel was performed using Quant Array software (Packard Biochip), and graphs were generated in Microsoft Excel. The average signal values were taken from 6 spots on 3 slides processed in parallel.

7. Results

The immobilization reactivity was found to vary depending on the wavelengths during the immobilization procedure (Figure 1). The signal intensity was 2~7-fold greater for the UV-irradiated array with central wavelength of 280 nm than for the corresponding other wavelength controls. Thus 280 nm was illustrated to be the most efficient wavelength for immobilization of DNA.

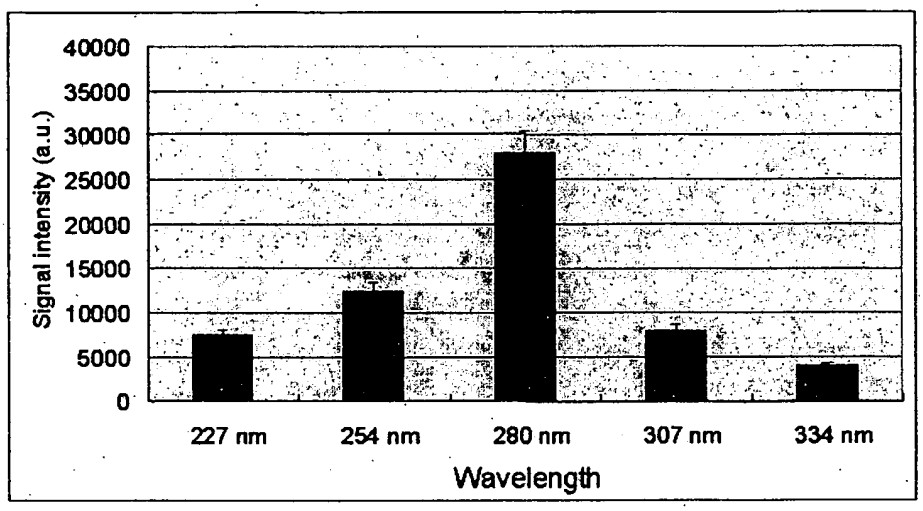

Figure 1. Effect of UV-irradiation on hybridization efficiency. Signal intensities produced upon immobilization of HEX-labeled oligonucleotide probe at 0.6 J/cm^2 of UV-irradiation. The error bars indicate standard deviation.

8. Jacobsen et al. and Zimlich et al. teach a method of immobilizing a biomolecule on a carrier comprising the steps of spotting a solution of the biomolecule on the carrier and irradiating the carrier spotted with the solution of the biomolecule with a light. However, the method of Jacobsen et al. irradiates a light whose wavelength is in the range from UV to visible light (see claim 1 of Jacobsen et al.). In the method of Zimlich et al., the wavelength of the light to be irradiated is within the range of 200 to 290nm (see claim 6 of Zimlich et al.) and specifically, 254nm (see column 4, line 44 of Zimlich et al.). Namely, in Jacobsen et al. and Zimlich et al., using an ultraviolet light of 280nm and its advantageous effect are neither disclosed nor suggested. On the other hand, as shown in the data of Table 1, the method of the present invention has an unexpected advantageous effect that biomolecules can be efficiently immobilized by irradiating an ultraviolet light of 280nm. Therefore, I believe that the present invention is neither anticipated nor obvious over the cited documents.

9. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States codes and that such willful, false statements may jeopardize the validity of the application or patent issuing therefrom.

Dated: 25/August/2006 By: Naoki Kimura

Naoki KIMURA